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Sorghum mutation breeding for tolerance to water deficit under climate change

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This study aimed at creating genetic variability by induced mutagenesis in farmers' preferred sorghum variety (ICSV1049) to breed mutant lines for water deficit tolerance. Sorghum seeds were irradiated by gamma rays and sown as one panicle-to-one progeny method. Putative lines M5 (143) and parent were screened under water deficit stress. Data analysis showed that leaf senescence (LS) was positively correlated to relative water content (RWC), panicle weight (PaWt), grain weight (GrWt) and chlorophyll content 13 days after water deficit application (SPAD II). Semi-dwarf trait (SDwf) with plants height (Ht)<100 cm were observed among 3.38% of lines, while 13.5% exhibited early maturity (<90 days). The leaves of 87.3% of lines were semi-erectile. Averaged overall lines, mutation has reduced date to flowering (DaFl), date to grain maturity (DaMa) and LS at 9.2, 4.1 and 8.1% compared to the parent, respectively. However, SPAD I (chlorophyll content first day of water deficit application), SPAD II, RWC, GrWt, PaWt and Ht were increased at 30.8, 40.5, 36.5, 22.2, 37.5 and 9.3%, respectively. Based on the results, seven mutant lines exhibited tolerance to water deficit.

Key words: Mutagenesis, genetic variability, drought-tolerance.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the major cereal crops in the world. It is the fifth most cultivated dry cereal after wheat, maize, rice and barley and the second most cultivated in Africa after maize (Ng'uni et al., 2011). It is a staple food crop for millions of African farmers living in the semi-arid tropics (Dora et al., 2014). However, sorghum cultivation is affected by drought, a situation which could become severe in sub-Saharan Africa in the context of climate change. Water deficit caused by drought is the most severe environmental limitation to sorghum grain yield during the entire crop production period (Sánchez-Blanco et al., 2002). Due to population growth (3%) in Africa, the core challenge for agriculture in Africa would be to increase food production under changing climatic conditions.

Drought events can occur at any stage of sorghum

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growth but three stages are identified as critical phases sensitive to water deficit (Menezes et al., 2015). The growth stage 1 (GS1) corresponds to the vegetative phase, the growth stage 2 (GS2) corresponds to the pre-flowering phase with panicle initiation at flowering and the growth stage 3 (GS3) corresponds to the post-floral phase with filling and physiological maturity of the grains. Stay-green in sorghum is one of the reliable traits related to drought tolerance. Traits associated with pre- or post-floral water deficit resistance in sorghum also involve relative water content (RWC) and leaf senescence (Sakhi et al., 2014). The most sustainable ways to mitigate adverse effects of drought on sorghum production are field irrigation and provision of drought-tolerant varieties to farmers. Unfortunately, farmers in developing countries cannot afford irrigation facilities. Therefore, the development of drought-tolerant sorghum varieties is the most promising option to assist African farmers in adapting to drought. The strategy for development of new crop genotypes for drought tolerance could be to create variation within the gene pool. Genetic variability in traditional sorghum varieties is very low in Burkina Faso, around 4.5% of the genetic variability between agro-ecological zones and 5.8% between villages in the same zone (Kondombo-Barro, 2010). Mutation induction has been proven to be an effective method to increase genetic variability in crops. Induced mutagenesis in crop varieties preferred by farmers is a promising strategy to improve agronomic traits such as tolerance to water stress. Genetic variability created through mutagenesis is important for sustainable agriculture (Griggs et al., 2013). According to International Atomic Energy Agency (IAEA) database (http://mvgs.iaea.org), there are more than 3,300 officially released mutant varieties of 170 different species in more than 60 countries around the world that not only increase biodiversity but also provide material for plant breeding (Jankowicz-Cieslak et al., 2017). Mutation induction can be carried out using chemical or physical mutagens (Shahab et al., 2018). Some of the agronomic traits generated as result of mutation induction are: increasing 3-Deoxyanthocyanidin accumulation in sorghum leaves (Petti et al., 2014), dwarfism, early flowering, high protein digestibility and high lysine content which have been widely used in sorghum breeding (Oria et al., 2000). The aim of this study was to develop drought-tolerant mutants in a farmer-preferred sorghum variety (ICSV1049) for adaptation to water deficit that limits cereal production in sub-Saharan Africa.

MATERIALS AND METHODS

Study sites and genetic materials

A survey on adoption and dissemination of sorghum varieties from participatory breeding in Burkina Faso was conducted in partnership between the Institute for the Environment and Agricultural Research (INERA) and the Centre for International Cooperation in Agronomic Research for Development (CIRAD). Based on results from the survey, sorghum varieties preferred by farmers were identified (Sanou et al., 2014), including variety ICSV1049. Some traits of this variety include: 115-120 days to maturity, plant height ranging from 1.80 to 2.10 m, white grain colour and semi erectile leaf. The dry seeds of this variety were irradiated with gamma rays (^{60}Co) at doses of 200, 300 and 400 Gy at the Center for the Application of Isotope and Radiation Technology, National Nuclear Energy Agency (BATAN), Jl. Cinere Pasar Jumat, Jakarta, Indonesia. The trials were conducted in two localities, namely the eastern region (Kouaré Research Station) (11° 95' 03'' N and 0° 30' 58'' E) where mutant lines screening up to M4 generation was carried out and a selection of potential drought-tolerant mutants was achieved after terminal stress. Screening of M5 mutant lines was conducted in the central region (Kamboinsé Research Station) (12°28 N, 1° 32 W) for artificial water deficit application to minimize the environmental effect on the genotypes.

Both research stations are located in the North-Sudanian agroecological zone (latitude 11°30"–13°) with rainfall between 750-1000 mm per year. The soils are mostly revived tropical ferruginous types on Kamboinsé Station and sandy-loam, tropical, and ferruginous on Kouaré Station.

Generation of mutant progenies and selection of potential drought-tolerant M4 mutant lines

The irradiated seeds and control were sown and M1 panicles were harvested and planted as M2 panicle-to-one progeny. Forty of the M2 seeds from each M1 panicle were planted as a head row. Three panicles from each row were bagged before anthesis. To prevent redundancy of mutations, only one fertile plant from each M2 head row were selected to produce M3 seeds according to Xin et al. (2008). The M3 families were repeatedly evaluated for phenotypes distinctive from wild-type ICSV1049. Thus, the phenotypes were organized into tillering types, plant height, leaves vigor, panicle shapes and seeds size from ascend stage to grains physiological maturity. 394 lines were selected on the basis of the phenotypes described earlier and confirmed in the next generation. A field-trial was conducted during the cropping season in 2017 at Kouaré Research Station to evaluate 394 putative mutant lines at the M4 generation and their parent for tolerance to the end of season drought. The planting was done on 18th August so that the bloom stage coincided with the end of rainfall. Each line was planted on row 2.7 m length, 0.3 m between planting hills and 0.7 m between rows. The experimental design was an alpha lattice design plot using 15 blocks and each block contained 20 genotypes with two replications.

The field was weeded three times. Mineral fertilizer of 100 kg ha^{-1} of NPK (12-24-12) was applied to the plots at sowing and 50 kg ha^{-1} of urea was applied at the booting growth stage. The amount of rainfall recorded from planting to the harvest (18th August to 15th December, 2017) was 201.5 mm corresponding to 10 rain events or 23.4% of total rainfall (860 mm) recorded in 2017 on Kouaré Station.

The selection of drought-tolerant lines was made in under field conditions based on productivity per line including phenotypic traits such as tiller number, panicle filling, grains quality, number of leaves per plant and leaf vigour. A total of 143 mutant progenies M5 were selected for screening under water deficit in controlled conditions.

Screening of M5 mutant lines under soil water deficit

The potential drought tolerant lines which exhibited different

Table 1. Frequency of induced traits observed in sorghum M5 mutant lines.

Phenotype description	Abbreviation	Number of mutant lines	Frequency (%)
Semi-erectile leaves (normal)	Nm	103	87.28
Late maturity (>90 days)	LMa	102	86.44
Single stalked	Sst	92	77.96
Multiple tillers	Mtl	26	22.03
Early maturity (<90 days)	EMa	16	13.55
Erect leaves	ErL	15	12.71
Semi-dwarf (<100 cm)	SDwf	4	3.38

Table 2. Effect of mutation on the reduction or increasing of parameters.

Parameter	Means of parameters								
	SPAD I	SPAD II	DaFl	DaMa	RWC	LS	GrWt	PaWt	Ht
ML	44.1	25.3	69	93	46.7	86.3	23.1	55	126.1
Wt	33.7	18	76	97	34.2	94	18.9	40	115.3
Gr/Re (%) P<0.0001	30.8	40.5	9.2	4.1	36.5	8.1	22.2	37.5	9.3

ML = mutant line, Wt= wild type, Ht= plant height (cm), DaFl= flowering date (days), DaMa= maturity date (days), RWC= relative water content (%), LS= leaf senescence (%), GrWt= grain weight (g) and PaWt= panicle weight (g), Gr (%): percentage of increased traits, Re (%) = percentage of reduced traits.

morphological traits of the parent were selected and confirmed by screening under water deficit in controlled conditions during the dry season of 2018. The experiment was conducted at Kamboinsé research station (143 mutant lines and one control were screened). The experimental design used was an alpha lattice square with 12 blocks and 12 genotypes per block using three replications. Each genotype was sown on row of 1.5 m length. The spacing of planting hills within single and between rows, plot fertilization and weeding were carried out as described previously. After planting, watering was performed every three days with tap water installed around the field. Sorghum seedlings were thinned at 14 days after sowing to get one plant per hill.

Water deficit stress was applied to sorghum plants by cessation of irrigation 65 days after the sowing (DAS) until harvest.

Data collection and statistical analyses

From each surviving plant, the following parameters were measured: (1) panicle weight per plant (PaWt); (2) grain weight per plant (GrWt), (3) mature plant height (Ht); (4) days to flowering (DaFl); (5) days to Maturity (DaMa); (6) the chlorophyll content 65 days after sowing (DAS) (SPAD I) and 13 days after the stress (DASt) or 78 DAS (SPAD II) using the chlorophyll meter (SPAD 502 Plus); (7) leaf senescence (LS) scored 14 DASt using a scoring scale (Sakhi et al., 2014); (8) relative leaf water content (RWC %) 7 DASt (Saddam et al., 2014). The phenotypes were grouped into tillers number, dwarf plants, early maturity, late maturity, single stalked, erectile leaves compared to the parent characters. Growth percentage/reduction percentage (Gr/Re) was calculated as:

$$\text{Gr or Re (\%)} = ((\text{Wt value} - \text{ML value}) \times 100)/\text{Wt value}.$$

Statistical analyses were carried out using Statistical Analysis System (SAS, 9.1, 2 Institute, Cary, NC). Analysis of variance (ANOVA) was used to determine significance based on P-value. Means were separated using Newman Keuls Multiple Range test and differences between sorghum lines traits were considered significant levels of 5% ($P < 0.05$). The correlation coefficient between traits and genotypes clustering were analysed using R x 64 3.5.2 software.

RESULTS

Induced traits in M5 mutant lines

Selection of sorghum mutants is based on phenotypes observed by comparison of putative mutants with the parent variety (ICSV1049). Upon exposition of 143 M5 lines to soil water deficit, 118 lines and the parent survived and the semi-erect leaves, late maturity and single stalked were the most frequently observed phenotypes (Table 1). Some agronomic traits such as tiller number, plant height, leaf aspect and grain maturity were affected by gamma radiation (Table 1). As expected, variation was higher in the M5 population screened under water deficit compared to control population.

ANOVA showed significant differences ($P < 0.0001$) between mutant lines (ML) and wild type (Wt) for all measured parameters. Averaged overall lines, date to flowering (DaFl), date to grain maturity (DaMa) and leaf senescence (LS) were reduced at 9.2, 4.1 and 8.1% compared to the parent, respectively. However, SPAD I, SPAD II, RWC, GrWt, PaWt and Ht were increased at 30.8, 40.5, 36.5, 22.2, 37.5 and 9.3%, respectively due to mutation effect (Table 2).

Table 3. Pearson correlation between measured traits of Sorghum M5 population screened at Kamboinsé, 2018.

Correlation	Ht	SPAD I	SPAD II	DaFl	DaMa	RWC	GrWt	PaWt	LS
Ht	1								
SPAD I	-0.15	1							
SPAD II	0.04	0.04	1						
DaFl	0.28*	-0.51**	0.17*	1					
DaMa	0.18*	-0.5**	0.18*	0.75**	1				
RWC	0.008	-0.13*	0.08	0.03	-0.002	1			
GrWt	0.04	0.31*	0.16*	-0.20*	-0.28*	0.25*	1		
PaWt	0.01	0.41*	0.21*	-0.27*	-0.32*	0.25*	0.76**	1	
LS	0.08	0.16*	0.24*	-0.09	-0.06	0.57**	0.36*	0.39*	1

Ht = Plant height (cm), SPAD I = chlorophyll content at first day of water deficit application (µmol/mg), SPAD II = chlorophyll content at 13 days after water deficit application (µmol/mg), DaFl = flowering delay (days), DaMa = physiological maturity delay (days), RWC = relative water content (%), GrW t= grains weight (g), PaWt = panicle weight (g), LS = leaf senescence (%). ** = highly significant difference at 5% threshold, * = significant difference at 1% threshold.

Table 4. Clustering of mutant lines and control according to LS.

Number of genotypes with means range (%)					
C1	C2	C3	C4	P value	CV (%)
105 (64.3-100)	6 (52.6-61.7)	5 (25.2-46.4)	3 (11.3-20)	<0.0001	11.74

CV: Coefficient of variation.

Correlations between measured parameters

Panicle weight (g) had high significance and positive correlation with grain weight (Table 3). Leaf senescence and relative water content were significantly and positively correlated (R^2=0.57%). Panicle and grain weight were also correlated with leaf senescence, SPAD I, SPAD II and RWC. The correlation between weight of panicle, weight of grains and leaf senescence is relevant to the leaf senescence effect on grain yield. However, there was a negative correlation between LS, DaFl and DaMa (Table 3).

RWC is negatively correlated to SPAD I and positively correlated with leaf senescence while plant height was positively correlated to all the characters except SPAD I which indicates that these traits do not evolve in the same direction as the SPAD I. There is a negative correlation between DaFl, MaDa and grain yield (Table 3).

Clustering and selecting of best mutant lines for tolerance to water deficit

Analysis of variance of LS showed that there was a significant difference (P < 0.0001) between the mutant lines. Therefore, genotypes were grouped with statistically identical leaf senescence values. Thus, sorghum mutants were classified into 4 clusters (Table 4). Mutant lines with average leaf senescence between 64.3 and 100% (C1) were 105 including the parent. The next cluster made up of 6 lines with average leaf senescence between 52.6 and 61.7% (C2) followed by cluster (C3) with 5 lines and an average leaf senescence around 25.2 to 46.4%. The last cluster (C4) consisting of 3 lines had an average leaf senescence around 11.3 to 20%. The lowest percentages of LS were recorded in C3 (LS average~36%) and C4 (LS average~16.38%) corresponding to the scale 4 and 2, respectively. The highest average SPAD I value was recorded with mutant lines found in cluster C3 (44.6 µmol/mg) and C1 (44.2 µmol/mg) compared to the others clusters. However, the coefficient of variation (<15%) indicates that there is low variability of chlorophyll content at the beginning of the application of soil water deficit. The highest values of SPAD II were recorded at 33.8 µmol/mg in C4 and there was high variability in chlorophyll content (SPAD II) after the application of soil water deficit (CV>15%). The highest grain and panicle weights were recorded with clusters C3 and C4 clusters and the lowest were recorded with C1 and C2. There was a significant difference (P<0.0001) between the traits within the cluster

Table 5. Means for different traits related to leaf senescence clustering.

Trait	C1			C2			C3			C4		
	Means	CV %	P-value	Means	CV %	P-value	Means	CV %	P-value	Means	CV %	P-value
Ht (cm)	126.6	10.8	<0.0001	137	10.8	0.29	115.5	9.7	0.02	104.2	17.5	0.54
SPAD I (µmol/mg)	44.2	13.5	<0.0001	40.6	12.2	0.13	44.6	14.4	0.04	42.1	15	0.39
SPAD II (µmol/mg)	24.7	26.3	<0.0001	29.8	20.3	0.02	26.4	31.2	0.16	33.8	19.5	0.26
DaFl (days)	69	7	<0.0001	71	6.2	0.28	66	7.7	0.18	70	8.3	0.84
DaMa (days)	93	2.4	<0.0001	94	2.9	0.83	95	4.4	0.06	94	5.5	0.95
RWC (%)	43.5	23.5	<0.0001	62.8	18.4	0.26	69.3	13.1	0.19	76.2	7.5	0.01
GrWt (g)	21.6	32.9	<0.0001	28.2	16.8	0.03	35.8	12.9	0.01	38.4	10.4	0.2
PaWt (g)	50.2	34.5	<0.0001	68.1	25.7	0.006	104.6	19.1	0.001	97.06	23.8	0.45

Ht= Plant height, SPAD I and SPAD II, chlorophyll content at initial day of water deficit application and 13 days after water deficit application, DaFl= flowering delay, DaMa= physiological maturity delay, RWC= relative water content, GrWt= grains weight, PaWt= panicle weight, LS= leaf senescence, CV = coefficient of variation.

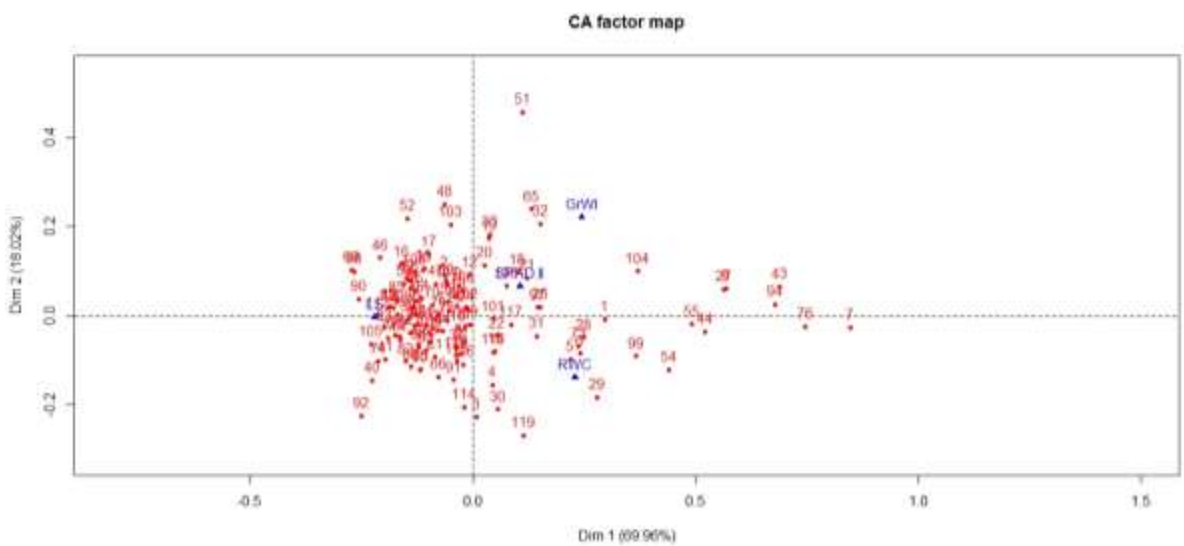

Figure 1. Detection of mutant lines with water deficit tolerance traits. The numbers represent the mutant lines.

C1. But no significant difference was observed within the other clusters (P>0.05) except C3 where PaWt exhibited a significant statistical difference (P<0.05) (Table 5). Tables 4 and 5 indicated that sorghum tolerant mutants to water deficit could be selected inside C3 and C4.

According to LS, RWC, SPADII and GrWt which are the best parameters of tolerance to water deficit, seven promising water deficit tolerant mutants were selected. This selecting was based on the mutants with high values of RWC, SPAD II, GrWt and low values of LS (Figure 1). Based on the analysis outputs, the best performing mutants under water deficit conditions were ICM5_6, ICM5_104, ICM5_76, ICM5_3, ICM5_30, ICM5_15 and ICM5_105. They are distinguished from other mutants and parent by high relative water content (between 61 - 83%), high SPAD II (21 – 37 µmol/mg) and GrWt (28-54 g) with the lowest LS (10.6-39.9%) while RWC, SPAD II, GrWt and LS of the parent were 34%, 18 µmol/mg, 18.9 g and 94%, respectively (Figure 2).

DISCUSSION

Mutation induction is a powerful tool that plant breeders use to create genetic variability. That variability can be exploited to select desired traits. Mutagens can affect all parts of the plants by either decreasing plants height or increasing it relative to the parent. Their effect can shorten or extend the plant cycle. In plants exposed to mutagens, morphological abnormalities and reduced

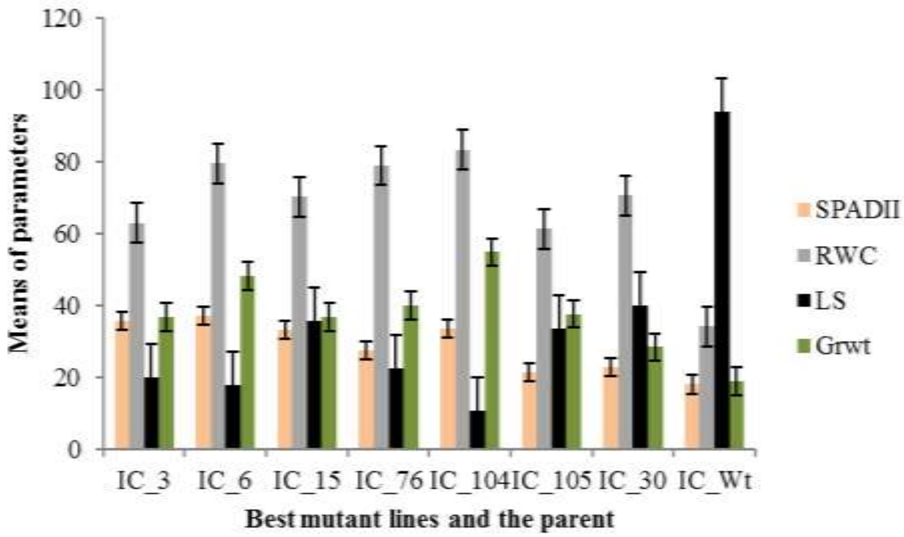

Figure 2. Water deficit tolerant mutant lines.

growth have been observed and attributed to oxidative stress (Singh, 2003) or deleterious mutational effects (Valluru et al., 2019). The mutagenesis affected some agronomic traits in millet such as reduction in plant height compared to the control (Ambli and Mullainathan, 2014). The results of this study showed that the overall height of plant mutant and grain yield were higher than that of the parent. These results support findings by previous studies (Burow et al., 2014) in which mutation increased sorghum plant height and grain yield. However, reduction in plant height was observed in mutagenized rice (Talebi et al., 2012), rapeseed and mustard (Javed et al., 2003). The present results together with those cited confirm that induced mutation using gamma rays can play an important role in the genetic variability induction within plant architecture.

The positive correlation between weights of panicle and grains to SPAD I, SPAD II and RWC suggests that these traits can be simultaneously selected and may be used as selection criteria for tolerance to soil water deficit. Negative correlation between RWC and SPAD I suggest that it would be difficult to select drought tolerant plants at the beginning of water deficit application based on these two parameters. The positive correlation between RWC and LS indicates that mutants which accumulate sufficient water in their leaves are those which have a slower leaf age. Therefore, genetic improvement of RWC also implies genetic improvement of LS. RWC is a useful trait for plants to mitigate the effects of drought at the reproductive stage. Negative correlation between delay flowering, delay maturity and grain yield implies that early mutants have no significant difference in yield compared to late mutants. These results disagree with those obtained by previous studies (Menezes et al., 2015) reporting that there is a positive correlation between productivity and maturity in sorghum grain under water deficit. Water deficit tolerance is the capacity of plants to support water deficit while keeping suitable physiological activities to safeguard cellular and metabolic integrity at tissue and cellular level (Xiong et al., 2006). Plant leaf senescence is considered as a post-flowering drought stress symptom (Burke et al., 2013). Green plants such as sorghum have two options for maintaining a high tissue water status during periods of soil moisture deficit, either by decreasing water loss due to transpiration or by increasing water uptake (Devnarain et al., 2016). Leaf senescence reduces seriously the source-sink translocation from leaves to grain (Krupa et al., 2017). In the present study, drought scoring based on LS was significantly higher for the wild type accession. Based on some reports (Ji et al., 2010) the results of this study on PaWt and GrWt revealed that soil water deficit affects grain number and weight. The decrease in quantitative traits such as yield of some mutant lines may be attributed to the physiological disruption or chromosomal deterioration caused to plant cells by the mutagen gamma ray (Thilagavathi and Mullainathan, 2011). Relative water content designates the metabolic activity in tissues and used as the most meaningful index for dehydration tolerance. RWC ranged between 85 and 95% and a critical reduction of less than 50% could cause tissue death (Vinodhana and Ganesamurthy, 2010). From the results of this study, some sorghum mutant lines were able to maintain RWC above 60% for 14 days during soil water deficit. Previous studies showed that maintenance of a relatively high RWC during mild drought is indicative of drought tolerance (Colom and Vazzana, 2003).

Sorbitol treatments to simulate drought-induced osmotic stress in sorghum cell suspension cultures showed that sorbitol raised an overall increase in secretion of 92 proteins that were differentially expressed in response to sorbitol-induced osmotic stress (Ngara et al., 2018). So, additional molecular studies on developed sorghum mutant lines would allow identifying protein or genes of interest via biotechnological or marker assisted breeding strategies with the prospect to combine them in one line for more performance.

Conclusion

Late drought is the most limiting factor in sorghum production. The results showed that induced mutation is a suitable tool to create genetic variability for selecting drought tolerant mutants. Further evaluation of those mutants to confirm their tolerance and stability under water deficit conditions would be useful. So, multi-local tests on other experimental sites will be conducted in the coming years to evaluate the agronomic performance of the best lines, taking into account genotype-environment interaction. In addition to leaf senescence and relative water content already recommended in phenotyping for drought-tolerance, the chlorophyll content 13 days after water stress application should also be considered as phenotypic trait in similar studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ambli K, Mullainathan L (2014). Induced physical and chemical studies in M1 generation of pearl millet (*Pennisetum typhoides*) (Burn.) Stapf.Var.co (cu)-9. International Journal of Recent Scientific Research 5(10):1806-1809.

Burke JJ, Chen J, Burow G, Mechref Y, Rosenow D, Payton P, Xin Z, Hayes CM (2013). Leaf dhurrin content is a quantitative measure of the level of pre- and post-flowering drought tolerance in sorghum. Crop Science 53(3):1056-1065. https://doi.org/10.2135/cropsci2012.09.0520.

Burow G, Xin Z, Hayes C, Burke J (2014). Characterization of a Multiseeded (msd1) Mutant of Sorghum for Increasing Grain Yield. Crop Science 54:1-8. https://doi.org/10.2135/cropsci2013.08.0566.

Colom M, Vazzana C (2003). Photosynthesis and PSII functionality of drought-resistant and drought-sensitive weeping lovegrass plants. Environmental and Experimental Botany 49(2):135-144. https://doi.org/10.1016/s0098-8472(02)00065-5.

Devnarain N, Crampton BG, Chikwamba R, Becker JVW, O'Kennedy MM (2016). Physiological responses of selected African sorghum landraces to progressive water stress and re-watering. South African Journal of Botany 103:61-69. https://doi.org/10.1016/j.sajb.2015.09.008

Dora SVVN, Polumahanthi S, Sarada MN (2014). Efficient callus induction protocol for *Sorghum bicolor*. Asian Journal of Plant Science and Research 4(3):14-21.

Griggs D, Smith MS, Gaffney O, Rockstrom J, Ohman MC, Shyamsundar P, Steffen w, Glaser G, Kanie N, Noble I (2013). Sustainable Development Goals for People and Planet. Nature 495(7441):305-307.

Jankowicz-Cieslak J, Thomas HT, Jochen K, Bradley JT (2017). Biotechnologies for Plant Mutation Breeding, Protocols; Library of Congress Control Number; © International Atomic Energy Agency ISBN 978-3-319-45019-3.

Javed MA, Siddiqui MA, Khan MKR, Khatri A, Khan IA, Dahar NA, Khanzada MH, Khan R (2003). Development of high yielding mutants of *Brassica campestris* L. cv. Toria selection through gamma rays irradiation. Asian Journal of Plant Sciences 2(2):192-195. http://dx.doi.org/10.3923/ajps.2003.192.195.

Ji X, Shiran B, Wan J, Lewis DC, Jenkins CLD, Condon AG, Richards R, Dolferus R (2010). Importance of pre-anthesis anther sinks strength for maintenance of grain number during reproductive stage water stress in wheat. Plant Cell Environment 33:926-942. https://doi.org/10.1111/j.1365-3040.2010.02130.x.

Kondombo-Barro PC (2010). Agromorphological and genetic diversity of local sorghum varieties [*Sorghum bicolor* (L.) Moench] from Burkina Faso. Elements of local genetic resources valorisation. PhD thesis in genetic and plants breeding. University of Ouagadougou.

Krupa KN, Ningaraj D, Shashidhar HE, Harinikumar KM, Manojkumar HB, Subhash B, Vinayak T (2017). Mechanisms of Drought Tolerance in Sorghum: A Review. International Journal of Pure and Applied Bioscience 5(4):221-237. http://dx.doi.org/10.18782/2320-7051.2845.

Menezes CB, Saldanha DC, Santos CV, Andrade LC, Júlio MPM (2015). Evaluation of grain yield in sorghum hybrids under water stress. Genetics and Molecular Research 14(4):12675-12683. http://dx.doi.org/10.4238.

Ngara R, Ramulifho E, Movahedi M, Chivasa S, Brown A, Shargie NG (2018). Identifying differentially expressed proteins in sorghum cell cultures exposed to osmotic stress. Scientific Reports 8:8671. 10.1038/s41598-018-27003-1.

Ng'uni D, Geleta M, Johansson E, Fatih M, Bryngelsson T (2011). Characterization of the Southern African sorghum varieties for mineral contents : Prospects for breeding for grain mineral dense lines. African Journal of Food Science 5:436-445.

Oria MP, Hamaker BR, Axtell JD, Huang CPA (2000). Highly digestible sorghum mutant cultivar exhibits a unique folded structure of endosperm protein bodies. Proceedings of the National Academy of Sciences 97(10): 5065-5070. https://dx.doi.org/10.1073%2Fpnas.080076297.

Petti C, Kushwaha R, Tateno M, Harman-Ware AE, Crocker M, Awika J, DeBolt S (2014). Mutagenesis Breeding for Increased 3-Deoxyanthocyanidin Accumulation in Leaves of Sorghum bicolor (L.) Moench: A Source of Natural Food Pigment. Journal of Agricultural and Food Chemistry 62(6):1227-1232. https://doi.org/10.1021/jf405324j.

Saddam S, Bibi A, Sadaqat HA, Usman BF (2014). Comparison of 10 sorghum (*Sorghum bicolor* (L.) Moench genotypes under various water stress regimes. Journal of Animal and Plant Sciences 24(6):1811-1820.

Shahab D, Gulfishan M, Khan AA, Vágvölgyi C, Ansari MYK (2018). Comparative Mutagenic Effectiveness and Efficiency of Physical and Chemical Mutagens in *Solanum melongena* L. Variety Pusa Uttam. Biomedical Journal of Scientific and Technical Research 7(4):6056-6060.

Sakhi S, Rehman S, Okuno K, Shahzad A, Jamil M (2014). Evaluation of Sorghum (*Sorghum bicolor*) Core Collection for Drought Tolerance: Pollen Fertility and Mean Performance of Yield Traits and Its Components at Reproductive Stage. International Journal of Agriculture and Biology 16(2):251-260.

Sánchez-Blanco MJ, Rodríguez P, Morales MA, Ortuño MF, Torrecillas A (2002). Comparative growth and water relations of *Cistus albidus* and *Cistus monspeliensis* plants during water deficit conditions and recovery. Plant Science 162(1):107-113. https://doi.org/10.1016/s0168-9452%2801%2900540-4.

Sanou A, Adam M, Brocke KV, Trouche G (2014). Bilan Thématique Programmé: Production agricole et sécurité alimentaire en Afrique de l'Ouest : Enquête sur l'adoption et la diffusion des variétés de sorgho issues de la sélection participative dans les régions Centre-Nord et Boucle du Mouhoun, 43p.

Singh SK (2003). Mutations in crop improvement. (ed. Singh, S. K). Plant breeding. Campus press, New Delhi, India.

Talebi AB, Talebi AB, Shahrokhifar B (2012). Identify the lethal dose of EMS and gamma radiation mutagenesis in rice MR219. International Proceedings of Chemical, Biological and Environmental Engineering, 48.

Thilagavathi C, Mullainathan L (2011). Influence of physical and chemical mutagens on quantitative characters of *Vigna mungo* (L. Hepper). International Multidisciplinary Research Journal 1(1):6-8.

Valluru R, Fernandes SB, Ferguson J, Brown P (2019). Deleterious Mutation Burden and Its Association with Complex Traits in Sorghum (*Sorghum bicolor*). Genetics 211(3):1075-1087. https://doi.org/10.1534/genetics.118.301742.

Vinodhana NK, Ganesamurthy K (2010). Evaluation of morpho-physiological characters in sorghum (*Sorghum bicolor* L. Moench) genotypes under post-flowering drought stress. Electronic Journal of Plant Breeding 1(4):585-589.

Xin Z, Wang ML, Barkley NA, Burow G, Franks C, Pederson G, Burke J (2008). Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population. BMC Plant Biology 8:103. https://doi.org/10.1186/1471-2229-8-103.

Xiong L, Wang R, Mao G, Koczan JM (2006). Identification of Drought Tolerance Determinants by Genetic Analysis of Root Response to Drought Stress and Abscisic Acid 1. Plant Physiology 142:1065-1074. https://doi.org/10.1104/pp.106.084632.

Talebi AB, Talebi AB, Shahrokhifar B (2012). Identify the lethal dose of EMS and gamma radiation mutagenesis in rice MR219. International Proceedings of Chemical, Biological and Environmental Engineering 48: 22–26.